

## Urine (Exfoliated Cell) RNA Purification Kit

Product # 22500

## Product Insert

Norgen's Urine (Exfoliated Cell) RNA Purification Kit provides a rapid method for the isolation and purification of total RNA from exfoliated cells that have been shed into the urine from the urinary tract. RNA biomarkers from exfoliated cells can be used as non-invasive tool for a number of diagnostic and research applications including the diagnosis and monitoring of bladder, kidney, or other urinary-tract cancers. The kit purifies all sizes of RNA, from large mRNA and ribosomal RNA down to microRNA (miRNA) and small interfering RNA (siRNA). The RNA is preferentially purified from other cellular components such as proteins, as well as from the contaminating species found in urine such as glucose and salts, without the use of phenol or chloroform. The purified RNA is of the highest integrity, and can be used in a number of downstream applications including real time PCR, reverse transcription PCR, Northern blotting, RNase protection and primer extension, and expression array assays.

### Norgen's Purification Technology

Purification is based on spin column chromatography using Norgen's proprietary resin as the separation matrix. The RNA is preferentially purified from other cellular components such as proteins without the use of phenol or chloroform. The process involves first lysing the exfoliated cells with the provided Lysis Solution (please see the flow chart on page 4). Ethanol is then added to the lysate, and the solution is loaded onto a spin-column. Norgen's resin binds RNA in a manner that depends on ionic concentrations, thus only the RNA will bind to the column while the contaminating proteins will be removed in the flowthrough or retained on the top of the resin. The bound RNA is then washed twice with the provided wash buffer in order to remove any remaining impurities, and the purified total RNA is eluted with the elution buffer. The purified RNA is of the highest integrity, and can be used in a number of downstream applications.

### Specifications

Kit Specifications	
Column Binding Capacity	50 µg
Volume of Urine Processed	1 – 50 mL
Maximum Input of Exfoliated Cells	1 x 10 <sup>6</sup>
Size of RNA Purified	All sizes, including small RNA (<200 nt)
Time to Complete 10 Purifications	20 minutes
Average Yield	~ 1 µg RNA per 1 x 10 <sup>5</sup> cells (Varies due to cell density sample)

### Advantages

- Fast and easy processing using rapid spin-column format
- Isolate total RNA, from large rRNA down to microRNA (miRNA)
- RNA can be isolated and detected from as little as 100 exfoliated cells
- Isolate high quality total RNA from urine
- No phenol or chloroform extractions

## Kit Components

Component	Product # 22500 (20 samples)
Lysis Solution	9 mL
Wash Solution	10 mL
Elution Buffer	6 mL
Micro Spin Columns	20
Collection Tubes	20
Elution tubes (1.7 mL)	20
Product Insert	1

## Storage Conditions and Product Stability

All solutions should be kept tightly sealed and stored at room temperature. These reagents should remain stable for at least 1 year in their unopened containers.

## Precautions and Disclaimers

This kit is designed for research purposes only. It is not intended for human or diagnostic use.

Ensure that a suitable lab coat, disposable gloves and protective goggles are worn when working with chemicals. For more information, please consult the appropriate Material Safety Data Sheets (MSDSs). These are available as convenient PDF files online at [www.norgenbiotek.com](http://www.norgenbiotek.com).

## Customer-Supplied Reagents and Equipment

You must have the following in order to use the Urine (Exfoliated Cell) RNA Purification Kit:

- Benchtop microcentrifuge
- $\beta$ -mercaptoethanol
- 95 - 100% ethanol
- RNase-free microcentrifuge tubes
- 50 mL conical tubes
- Swinging bucket centrifuge

## **Working with RNA**

RNases are very stable and robust enzymes that degrade RNA. Autoclaving solutions and glassware is not always sufficient to actively remove these enzymes. The first step when preparing to work with RNA is to create an RNase-free environment. The following precautions are recommended as your best defense against these enzymes.

- The RNA area should be located away from microbiological work stations
- Clean, disposable gloves should be worn at all times when handling reagents, samples, pipettes, disposable tubes, etc. It is recommended that gloves are changed frequently to avoid contamination
- There should be designated solutions, tips, tubes, lab coats, pipettes, etc. for RNA only
- All RNA solutions should be prepared using at least 0.05% DEPC-treated autoclaved water or molecular biology grade nuclease-free water
- Clean all surfaces with commercially available RNase decontamination solutions
- When working with purified RNA samples, ensure that they remain on ice during downstream applications

## Flow Chart

Procedure for Purifying Urine RNA using Norgen's Urine (Exfoliated Cell) RNA Purification Kit

Collect Urine Sample

**SPIN**  Pellet exfoliated cells



Lyse cells using Lysis Solution.  
Add Ethanol



Bind to column

**SPIN** 



Wash twice with  
Wash Solution

**SPIN** 



Elute RNA with  
Elution Buffer

**SPIN** 

**Purified Total Urine RNA**

## Procedures

All centrifugation steps are carried out in a benchtop microcentrifuge. Various speeds are required for different steps, so please check your microcentrifuge specifications to ensure that it is capable of the proper speeds. All centrifugation steps are performed at room temperature. The correct rpm can be calculated using the formula:

$$RPM = \sqrt{\frac{RCF}{(1.118 \times 10^{-5}) (r)}}$$

where  $RCF$  = required gravitational acceleration (relative centrifugal force in units of g);  $r$  = radius of the rotor in cm; and  $RPM$  = the number of revolutions per minute required to achieve the necessary  $g$ -force.

## Protocol for Total RNA Purification from Exfoliated Cells in Urine

All centrifugation steps are carried out in a benchtop microcentrifuge at 14,000 x g (~ 14,000 RPM) except where noted. All centrifugation steps are performed at room temperature.

### Notes Prior to Use

- A variable speed centrifuge should be used for maximum kit performance. If a variable speed centrifuge is not available a fixed speed centrifuge can be used, however reduced yields may be observed.
- Ensure that all solutions are at room temperature prior to use.
- Prepare a working concentration of the **Wash Solution** by adding 20 mL of 95% ethanol (provided by the user) to the supplied bottle containing the concentrated **Wash Solution**. This will give a final volume of 30 mL. The label on the bottle has a box that may be checked to indicate that the ethanol has been added.
- Prepare an appropriate amount of Lysis Solution by adding 10  $\mu$ L of  $\beta$ -mercaptoethanol (provided by the user) to each 1 mL of Lysis Solution required.  $\beta$ -mercaptoethanol is toxic and should be dispensed in a fume hood.
- Cell pellets can be stored at  $-70^{\circ}\text{C}$  for later use or used directly in the procedure.
- Frozen pellets should be stored for no longer than 2 weeks to ensure that the integrity of the RNA is not compromised.
- It is important to work quickly during this procedure.
- The maximum input of urine per column is 50 mL or  $1 \times 10^6$  exfoliated cells.

### 1. Cell Lysate Preparation

- a. Transfer 30 mL of urine to a 50 mL conical tube. Centrifuge the samples in a swinging bucket centrifuge at 650 x g for 5 minutes. The maximum input of urine is 50 mL or  $1 \times 10^6$  cells per column. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

**Note:** For samples less than 1.5 mL, transfer urine to a micro centrifuge tube and centrifuge at 250 x g (~2,000 RPM) for 5 minutes to pellet the cells. Pour off the supernatant carefully so as not to disturb or dislodge the cell pellet.

- b. Add 350  $\mu$ L of **Lysis Solution** to the pellet. Lyse cells by vortexing for 15 seconds. Ensure that the entire pellet is completely dissolved before proceeding to the next step. Transfer the lysate to an RNase-free microcentrifuge tube.
- c. Add 200  $\mu$ L of 95 – 100% ethanol (provided by the user) to the lysate. Mix by vortexing for 10 seconds.

## 2. Binding to Column

- a. Assemble a column with one of the provided collection tubes.
- b. Apply the lysate with the ethanol onto the column and centrifuge for 1 minute.

**Note:** Ensure the entire lysate has passed through into the collection tube by inspecting the column. If the entire lysate volume has not passed, spin for an additional minute.

- c. Discard the flowthrough. Reassemble the spin column with its collection tube.

### Optional Step:

Norgen's Urine (Exfoliated Cell) RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional **On-Column DNA Removal Protocol** is provided in Appendix A for maximum removal of residual DNA that may affect sensitive downstream applications. This step should be performed at this point in the protocol.

## 3. Column Wash

- a. Apply 400  $\mu\text{L}$  of **Wash Solution** to the column and centrifuge for 1 minute.

**Note:** Ensure the entire wash solution has passed through into the collection tube by inspecting the column. If the entire wash volume has not passed, spin for an additional minute.

- b. Discard the flowthrough and reassemble the spin column with its collection tube.
- c. Repeat steps **3a** and **3b** to wash column a second time.
- d. Wash column a third time by adding another 400  $\mu\text{L}$  of **Wash Solution** and centrifuging for 1 minute.
- e. Discard the flowthrough and reassemble the spin column with its collection tube.
- f. Spin the column for 2 minutes in order to thoroughly dry the resin. Discard the collection tube.

## 4. RNA Elution

- a. Place the column into a fresh 1.7 mL Elution tube provided with the kit.
- b. Add 50  $\mu\text{L}$  of **Elution Buffer** to the column.
- c. Centrifuge for 2 minutes at **200 x g (~2,000 RPM)**, followed by a 1 minute spin at **14,000 x g (~14,000 RPM)**. Note the volume eluted from the column. If the entire 50  $\mu\text{L}$  has not been eluted, spin the column at 14,000 x g (~14,000 RPM) for 1 additional minute.

**Note:** A smaller volume of Elution Buffer may be used in order to obtain a more concentrated sample. A minimum volume of 20  $\mu\text{L}$  is recommended

## 5. Storage of RNA

The purified RNA sample may be stored at  $-20^{\circ}\text{C}$  for a few days. It is recommended that samples be placed at  $-70^{\circ}\text{C}$  for long term storage.

## Appendix A

### Protocol for Optional On-Column DNA Removal

Norgen's Urine (Exfoliated Cell) RNA Purification Kit isolates total RNA with minimal amounts of genomic DNA contamination. However, an optional protocol is provided below for maximum removal of residual DNA that may affect sensitive downstream applications. It is recommended that an RNase-free DNase I be used.

1. Prepare a working stock of 0.25 Kunitz unit/ $\mu\text{L}$  RNase-free DNase I solution according to the manufacturer's instructions. A 100  $\mu\text{L}$  aliquot is required for each column to be treated. Alternatively, dissolve or dilute stock DNase I in a reaction buffer (40 mM Tris pH 7.0, 10 mM  $\text{MgCl}_2$  and 3 mM  $\text{CaCl}_2$ , made RNase-free) to give a final concentration of 0.25 Kunitz unit/ $\mu\text{L}$ .
2. Perform the RNA isolation procedure up to and including "**Binding to Column**" (Steps 1 and 2 of protocol).
3. Apply 400  $\mu\text{L}$  of **Wash Solution** to the column and centrifuge for 2 minute. Discard the flowthrough. Reassemble the spin column with its collection tube.
4. Apply 100  $\mu\text{L}$  of the RNase-free DNase I solution prepared in Step 1 to the column and centrifuge at 14, 000 x g (~14 000 RPM) for 1 minute.

**Note:** Ensure that the entire DNase I solution passes through the column. If needed, spin at 14, 000 x g (~14 000 RPM) for an additional minute.

5. After the centrifugation in Step 4, pipette the flowthrough that is present in the collection tube back onto the top of the column.

**Note:** Ensure Step 5 is performed in order to ensure maximum DNase activity and to obtain maximum yields of RNA, in particular for small RNA species.

6. Incubate the column assembly at 25 - 30°C for 15 minutes.
7. Without any further centrifugation, proceed directly to "**Column Wash**" (Step 3 of protocol).

## Troubleshooting Guide

Problem	Possible Cause	Solution and Explanation
Poor RNA Recovery	Incomplete lysis of cells	Ensure that the appropriate amount of Lysis Solution was added to the exfoliated cell pellet.
	Column has become clogged	Do not exceed the recommended amounts of 50 mL of urine or $1 \times 10^6$ cells. The amount of starting material may need to be decreased if the column shows clogging below the recommended levels. See also "Clogged Column" below.
	An alternative elution solution was used	It is recommended that the Elution Buffer supplied with this kit be used for maximum RNA recovery.
	Ethanol was not added to the lysate	Ensure that the appropriate amount of ethanol is added to the lysate before binding to the column.
	Ethanol was not added to the Wash Solution	Ensure that 20 mL of 95% ethanol is added to the supplied Wash Solution prior to use.
	Low cell density in the sample	The cell number in different urine samples vary. While individuals with various diseases have >1000 exfoliated cells per mL of urine, a healthy male may have a number much lower than the 1000 cells per mL limit. It is possible that the total RNA isolated is not visible when resolved on an agarose gel or detected by spectrophotometry. In such cases, a larger input volume may be used. Alternatively, a more sensitive method such as BioAnalyzer or RT-PCR may be used for detection.
Clogged Column	Insufficient solubilization of cells	Ensure that the appropriate amount of Lysis Solution was added to the exfoliated cell pellet.
	Maximum number of cells exceeds kit specifications	Do not exceed the recommended amounts of 50 mL of urine or $1 \times 10^6$ cells.
	High amounts of genomic DNA present in sample	The lysate may be passed through a 25 gauge needle attached to a syringe 5-10 times in order to shear the genomic DNA prior to loading onto the column.
	Centrifuge temperature too low	Ensure that the centrifuge remains at room temperature throughout the procedure. Temperatures below 20°C may cause precipitates to form that can cause the columns to clog.

Problem	Possible Cause	Solution and Explanation
RNA is Degraded	RNase contamination	RNases may be introduced during the use of the kit. Ensure proper procedures are followed when working with RNA. Please refer to “ <i>Working with RNA</i> ” at the beginning of this user guide.
	Procedure not performed quickly enough	In order to maintain the integrity of the RNA, it is important that the procedure be performed quickly
	Improper storage of the purified RNA	For short term storage RNA samples may be stored at –20°C for a few days. It is recommended that samples be stored at –70°C for longer term storage.
	The cells are old	Older samples contain prematurely lysed cells which release RNase and can degrade RNA. Fresh urine samples are recommended.
RNA does not perform well in downstream applications	RNA was not washed twice with the provided Wash Solution	Traces of salt from the binding step may remain in the sample if the column is not washed twice with Wash Solution. Salt may interfere with downstream applications, and thus must be washed from the column.
	Ethanol carryover	Ensure that the dry spin under the Column Wash procedure is performed, in order to remove traces of ethanol prior to elution. Ethanol is known to interfere with many downstream applications.
Genomic DNA contamination	Large amounts of starting material used	Perform RNase-free DNaseI digestion on the RNA sample after elution to remove genomic DNA contamination (Appendix A).

Related Products	Product #
Total RNA Purification Kit	17200
Cytoplasmic & Nuclear RNA Purification Kit	21000
Leukocyte RNA Purification Kit	21200
microRNA Purification Kit	21300
100b RNA Ladder	15002
1kb RNA Ladder	15003

### Technical Support

Contact our Technical Support Team between the hours of 8:30 and 5:30 (Eastern Standard Time) at (905) 227-8848 or Toll Free at 1-866-667-4362.

Technical support can also be obtained from our website ([www.norgenbiotek.com](http://www.norgenbiotek.com)) or through email at [techsupport@norgenbiotek.com](mailto:techsupport@norgenbiotek.com).

3430 Schmon Parkway, Thorold, ON Canada L2V 4Y6  
 Phone: (905) 227-8848  
 Fax: (905) 227-1061  
 Toll Free in North America: 1-866-667-4362